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Two faces of the prokaryote concept

Summary. Bacteria had remained undefined when, in 1962, Roger Y. Stanier and C.B. van Niel published their famed paper “The concept of a bacterium.” The articulation of the prokaryote–eukaryote dichotomy was a vital moment in the history of biology. This article provides a brief overview of the context in which the prokaryote concept was successfully launched in the 1960s, and what it was meant to connote. Two concepts were initially distinguished within the prokaryote–eukaryote dichotomy at that time. One was organizational and referred to comparative cell structure; the other was phylogenetic and referred to a “natural” classification. Here, I examine how the two concepts became inseparable; how the prokaryotes came to signify a monophyletic group that preceded the eukaryotes, and how this view remained unquestioned for 15 years, until the birth of molecular evolutionary biology and coherent methods for bacteria phylogenetics based on 16S rRNA. Today, while microbial phylogeneticists generally agree that the prokaryote is a polyphyletic group, there is no agreement on whether the term should be maintained in an organizational sense. [*Int Microbiol* 2006; 9(3):163-172]

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The scandal of bacteriology

In their famed paper of 1962, “The concept of a bacterium”, Roger Y. Stanier (1916–1982) and Cornelis B. van Niel (1897–1985) emphasized that the nature and relationships of bacteria remained unresolved: “the abiding intellectual scandal of bacteriology has been the absence of a clear concept of a bacterium” [51]. Structurally speaking, there had been several enduring issues. Was it true that bacteria lacked a nucleus? How could one distinguish bacteria from viruses? What about blue-green algae? Did they also lack a nucleus? Stanier and van Niel explained that a satisfactory description of the difference between bacteria and blue-green algae on the one hand and viruses and nucleated cells on the other could be articulated only after the advances in microscopy, molecular biology, and genetics following the World War II. They

acknowledged that one of their predecessors, Edouard Chatton (1883–1947) [47 bis]) had also recognized that the cell structure of bacteria and blue-green algae was different from that of other organisms:

“It is now clear that among organisms there are two different organizational patterns of cells, which Chatton (1937) (sic) called, with singular prescience, the eukaryotic and prokaryotic type. The distinctive property of bacteria and blue-green algae is the prokaryotic nature of their cells. It is on this basis that they can be clearly segregated from all other protists (namely, other algae protozoa and fungi), which have eukaryotic cells” [51].

Since that time, Chatton’s “prophetic insight” has been often repeated, and indeed mythologized at the font of the prokaryote–eukaryote dichotomy both as an organizational

and a taxonomic distinction at the highest level [43]. As recent scholarship has shown, Chatton did not articulate the distinction (he wrote very little about it) and he certainly did not see it as the basis for taxa, nor did Stanier and van Niel when they revitalized the dichotomy in 1962 [43]. Moreover, many microscopists since the turn of the 20th century had also distinguished bacteria and blue-green algae from other organisms that possessed a nucleus. The nature of their arguments and the significance of the distinction differ between writers; it is important to differentiate them.

The meanings of Monera

Ernst Haeckel (1834–1919) was perhaps the first biologist to group bacteria and blue-green algae together as organisms that lacked a nucleus. In his *Generelle Morphologie der Organismen* (1866), he proposed that life-forms without a nucleus, which he called “monera,” were at the lowest stage of a third kingdom, Protista [20]. In biology textbooks today, both Protista and Monera are often presented as kingdoms. However, although the kingdom Monera is attributed to Haeckel, his concept differed from today’s in two fundamental ways: Haeckel was uncertain whether that group of organisms had a single origin or whether it originated independently several times; and his monera were conceived of as precellular entities supposed to lack all trace of the hereditary determinants present in other organisms.

The monera were born of Haeckel’s monist philosophy, which demanded the removal of explanatory boundaries between life and non-life. Monera complied as organisms that would have arisen spontaneously from inorganic materials. Most of the organisms Haeckel assigned to the group in 1866 were later shown to be either non-existent or belie the definition of monera as organisms without nuclei.

In his *The Wonders of Life*, published in 1904, Haeckel emphasized that bacteria and chromaceae (blue-green algae) were, however, true to the definition [21]. “The whole vital activity of the simplest monera”, he wrote, “especially the chromaceae is confined to their metabolism, and is therefore a purely chemical process, that may be compared to the catalysis of inorganic compounds” [20]. Bacteria and chromaceae simply lacked all traces of what Haeckel regarded as “the first, oldest, and most important process of division of labour”, the nucleus, which “discharges the functions of reproduction and heredity, and the *cytoplasm* of the cell body [which] accomplishes the metabolism, nutrition and adaptation” [21]. The difference between monera and any higher organism, he said was “greater in every respect than the difference between the organic monera and the inorganic crys-

tals. Nay, even the difference between unnucleated monera (as cytodes) and the real nucleated cells may fairly be regarded as greater still” [21].

Bacteria had been considered to be plants since the 1850s. They were often called *Schizomycetes* (fission fungi), and when they were grouped with blue-green algae they were called *Schizophyta* (fission plants), as Ferdinand Cohn (1828–1898) named them in 1875 [8,22]. Haeckel argued that bacteria had nothing in common with fungi, and that the only real comparison between chromaceae and plants was with the chromatophores (chromatella) (chloroplasts). Thus, he suggested that chloroplasts had evolved as “a symbiosis between a plasmodomonous green and plasmophagus not-green companion” [21].

Did bacteria and cyanophyceae possess a nucleus? Much depended on the definition of nucleus; and even observations were far from certain. Bacterial anatomical detail fell below the resolution of the light microscope of the early twentieth century. Although it did seem apparent that bacteria and cyanophyceae lacked a nucleus enclosed by a membrane, there were bacterial granules that stained with the same dyes used to stain chromatin of other cells. Thus, many researchers insisted, in direct opposition to Haeckel’s views, that bacteria possessed scattered nuclei, comparable to chromosomes of higher organisms. “If this identification is correct”, Edmund Beecher Wilson (1856–1939) wrote in 1900, in *The Cell in Development and Inheritance*, “such forms probably give us the most primitive condition of the nuclear substance, which only in higher forms is collected into a distinct mass enclosed by a membrane” [62].

Still, these matters remained unsettled [13,44,57]. In 1927, Edwin Copeland (1873–1964) argued that bacteria deserve a kingdom of their own when he wrote that “there is no other one thing so important in systematic biology as the fact that the grouping of organisms reflects and expresses their true relationships” [9]. In 1938, his son, Herbert Copeland (1902–1968) proposed that a kingdom be named with Haeckel’s term Monera, based on two assumptions: (1) that bacteria and blue-green algae are “the comparatively little modified descendants of whatever single form of life appeared on earth, and (2) that they are sharply distinguished from other organisms by the absence of nuclei” [10]. He therefore proposed four kingdoms: Monera, Protista, Planta, and Animalia.

Stanier’s and van Niel’s views were inconsistent. In 1941, they followed Copeland and assigned bacteria and blue-green algae to the kingdom Monera [50]. They also expanded the characterization of the group by adding two additional and equally negative criteria: the absence of plastids, and the absence of sexual reproduction. Then, in 1955, van Niel

renounced the Monera on the very same three grounds. New evidence based on electron microscopy seemed to suggest that bacteria possessed a nucleus, and that the photosynthetic bacterium *Rhodospirillum rubrum* possessed plastid-like entities [57]. Moreover, he argued, demonstrations of recombination in mixed cultures of bacteria by Lederberg and Tatum [27] demanded “a healthy scepticism with regard to the earlier belief that sexual phenomena do not occur among the bacteria” [57]. He concluded, “[t]hus it is clear that the criteria for a kingdom of organisms without nuclei do not apply to the bacteria and blue-green algae. This does not mean, however, that the notion of establishing a separate kingdom for these organisms should be abandoned” [57].

On this reasoning, one might suppose then that when the prokaryote concept was introduced in 1962, van Niel and Stanier would ultimately unveil Monera as a true kingdom. Importantly, they made no mention of Monera, and no declaration of a kingdom for bacteria and blue-green algae.

An organizational concept

Stanier and van Niel’s paper re-introducing the “prokaryote” and “eukaryote” was a sister to a famed paper by André Lwoff (1902–1994) who, in 1957, articulated the molecular and biochemical differences between the virus and the bacterium [28]. Since the 19th century, it had not been clear what the organizational difference was between these entities. Small obligate-parasitic bacteria of the rickettsial type seemed to be transitional between them. Indeed, in 1948, the editors of *Bergey’s Manual of Determinative Bacteriology* suggested the kingdom “Protophytes” for both bacteria and viruses [5]. Lwoff made the distinction hard and unambiguous: the virus was not a cell; it contained either RNA or DNA enclosed in a coat of protein; it possessed few if any enzymes; and it did not reproduce by division like a cell. Indeed, he found it difficult to conceive of any biological entities that could be transitional between a virus and a cell.

Stanier and van Niel’s concept of the bacterium took the next step to provide a definition that would “permit a clear separation of the bacteria *sensu lato* both from viruses and from other protists” [51]. Lwoff had recommended the terms “prokaryote” and “eukaryote”, i.e., those of his mentor Chatton [7], to Stanier, who first used the words in a paper written in French for *Annales de l’Institut Pasteur* in 1961 [48].

When Stanier and van Niel reintroduced the terms to English readers, their paper contained no retractions of van Niel’s previous claims of 7 years earlier—of nuclei in bacteria and of plastid-like entities in photosynthetic bacteria. Instead, van Niel’s paper of 1955 was simply not mentioned.

The organizational difference between prokaryote and eukaryote was made unequivocal; but, as they and other authors recognized, the prokaryote was still defined in negative terms [43]. Eukaryotes had a membrane-bound nucleus that divided by mitosis, a cytoskeleton, an intricate system of internal membranes, mitochondria that performed respiration, and, in the case of plants, chloroplasts. Bacteria (prokaryotes) were smaller; lacked all of these structures, and their nuclei reproduced by fission: “The principle distinguishing features of the procaryotic cell are: 1 absence of internal membranes which separate the resting nucleus from the cytoplasm, and isolate the enzymatic machinery of photosynthesis and of respiration in specific organelles; 2 nuclear division by fission, not by mitosis, a character possibly related to the presence of a single structure which carries all the genetic information of the cell; and 3 the presence of a cell wall which contains a specific mucopolysaccharide as its strengthening element” [51].

Just as there would be no transitional forms between viruses and bacteria, there would be no transitional entities between prokaryote and eukaryote. Stanier, Michael Doudoroff, and Edward Adelberg declared in the 2nd edition of *The Microbial World* (1963) that, “In fact, this basic divergence in cellular structure, which separates the bacteria and blue-green algae from all other cellular organisms, represents the greatest single evolutionary discontinuity to be found in the present-day world” [52]. Stanier and van Niel’s distinction was neither an evolutionary nor a taxonomic one—at least not as they drew it. In fact, their attitude toward an evolutionary-based classification of bacteria had taken a sudden change of course prior to 1962.

A world without evolution

The possibility of a “natural” classification of bacteria, one that reflected genealogical relationships, was intensely debated in the early 20th century [44]. In the *Origin of the Species*, Darwin had argued that “all true classification is genealogical; that community of descent is the hidden bond which naturalists have been unconsciously seeking, and not some unknown plan of creation, or the enunciation of general propositions, and the mere putting together and separating objects more or less alike” [12]. When constructing genealogical trees, as Darwin emphasized, comparisons of adaptive characters (those that were most closely related to the habits of the organisms) were the least useful because they would be relatively recent developments particular to the species or variety. Constructing phylogenetic trees required highly conserved ancient traits, ones that were far

removed from everyday life.

Such a phylogenetic classification among plants and animals could be based on comparative anatomy, comparative embryology, and an ever-expanding fossil record. But bacteria lacked complex morphological traits, developmental histories, and a fossil record. Bacteria did, of course, show enormous physiological or biochemical diversity, but it was difficult to discern which physiological traits were old and which were recent adaptations. By the early 1920s, many bacteriologists had given up on phylogeny and opted for a useful, reasonably stable, determinative classification based solely on utility, like the organization of library books [44].

Microbiologists, including van Niel, his mentor Albert Jan Kluyver (1888–1956), and van Niel's student, Stanier, had held out for a taxonomy that would reflect evolutionary relationships. In 1936, van Niel and Kluyver insisted that a phylogenetic classification be based, in the first instance, on increased morphological complexity [25]. Stanier and van Niel (1941) had reiterated the arguments for a phylogenetic classification in a cutting evaluation of *Bergey's Manual*, which they derided for rejecting a phylogenetic approach and for offering a poor definition of the bacterium [50]. At the famous Cold Spring Harbor Symposium of 1946, van Niel was at the cross-road [56]. Bacterial recombination was announced at that meeting, and bacteria were sent on their way to the center of (molecular) biology. The support by van Niel for the taxonomic schemes that he, Kluyver, and Stanier had proposed 10 years earlier had dwindled. He admitted: "Even for a general outline along phylogenetic lines, the available information is entirely inadequate. Much of this is, of course, the result of the paucity of characteristics, especially those of a developmental nature" [34]. Nonetheless, he avowed, "the search for a basis upon which a 'natural system' can be constructed must continue" [56].

In 1955, van Niel [35] finally rejected attempts at bacterial phylogenetics [57]. Three years earlier, Sergei Winogradsky (1856–1953) had also admitted that a phylogenetic classification was simply "impossible to apply to bacteria" [63]. They emphasized that the order of things in *Bergey's Manual*, in terms of species, genera, tribes, families, and orders, was only a façade. To avoid the delusion that it represented a natural ordering, the term "biotypes" was suggested instead of "species", and common names such as "sulfur bacteria", "photosynthetic bacteria" and "nitrogen-fixing bacteria" were proposed instead of Latin names with their phylogenetic implications.

Thus, by 1962, when Stanier and van Niel reintroduced the term "prokaryote," making no reference to the kingdom Monera, they had lost hope for a natural bacterial classification: "But even though we have become sceptical about the

value of developing formal taxonomic systems for bacteria..., the problem of defining these organisms as a group in terms of their biological organization is clearly still of great importance, and remains to be solved" [51]. In the first edition of *The Microbial World* (1957), Stanier, Doudoroff, and Adelberg reiterated that a natural classification of bacteria was impossible:

... the construction of the broad outlines of a natural system of bacterial classification involves much guesswork and affords the possibility for endless unprofitable disputes between the holders of different views about bacterial evolution. An eminent contemporary bacteriologist, van Niel, who is noted for his taxonomic studies on several groups of bacteria, has expressed the opinion that it is a waste of time to attempt a natural system of classification for bacteria, and that bacteriologists should concentrate instead on the more humble practical task of devising determinative keys to provide the easiest possible identification of species and genera. This opinion, based on a clear recognition and acceptance of our ignorance concerning bacterial evolution, probably represents the soundest approach to bacterial classification, but it has not gained universal acceptance [52].

Although they agreed that one could not have a phylogenetic classification of bacteria based on structure, Stanier and his colleagues were certain that bacteria were derived from one stock, that they shared a common ancestry. In *The Microbial World* (1963) they wrote: "All these organisms share the distinctive structural properties associated with the procaryotic cell (Chapter 4), and we can therefore safely infer a common origin for the whole group in the remote evolutionary past; we can also discern four principal sub-groups, blue-green algae, myxobacteria, spirochetes, and eubacteria, which seem to be distinct from one another [...]. Beyond this point, however, any systematic attempt to construct a detailed scheme of natural relationships becomes the purest speculation, completely unsupported by any sort of evidence" [52]. Thus, they concluded: "the ultimate scientific goal of biological classification cannot be achieved in the case of bacteria" [52].

A kingdom of prokaryotes

Stanier and van Niel's attitudes about taxa aside, the articulation of the prokaryote–eukaryote dichotomy in the 1960s had a major impact: biologists immediately assigned them to the rank of the kingdom Monera or superkingdoms: Prokaryotae and Eukaryotae [43]. In doing so, no microbiologist of the 1960s questioned that prokaryotes were a natural phylogenetic

ic group. If bacteria were polyphyletic, then the category prokaryote would have no evolutionary or phylogenetic meaning. When defined negatively, the taxon prokaryote might well be similar to the grouping “invertebrate,” which includes such diverse creatures as insects and worms.

Robert Whittaker changed his scheme in 1969 [61]. Ten years early, he had dropped Copeland’s Monera as a kingdom and, based on the heterotrophic nature of fungi, he distinguished them from plants and added the kingdom Fungi to Protista, Plantae, and Animalia [59,60]. Following Stanier and van Niel’s “argument of 1962, Whittaker proposed five kingdoms: Monera, Fungi (Mycota), Protists, Plants, and Animals” [61]. He did not question whether Monera was a monophyletic kingdom, but he was certain that, “[t]he three higher kingdoms [Plantae, Fungi and Animalia] are polyphyletic,” and he suspected that the same was true for Protista [61]. “Monophyly is a principal value of systematics,” he wrote, “but like other values is not absolute and will not always be followed to the sacrifice of other objectives” [61].

R.G.E. Murray, one of the members of the editorial board of *Bergey’s Manual*, was quick to suggest that the major structural or organizational differences between prokaryotes and eukaryotes be finally recognized as taxa at the highest level. When he wrote to Stanier in May 1962, stating that he could not understand the remarks they had made against a natural classification [36], Stanier replied:

I should certainly not object to setting up a separate kingdom for the prokaryotic microorganisms if such an operation would serve as a handy device for emphasizing the fundamental differences between these types and organisms that possess a eukaryotic cellular organization. All the introductory statement meant to imply is that both van Niel and I now consider detailed system building at the microbial level to be an essentially meaningless operation, since there is so very little information that can be drawn on for the purposes of phylogenetic reconstruction. For this reason I prefer to use common names rather than Latin ones for every bacterial group above the level of genus [49].

The monophyly of the Prokaryotes was not problematic for Murray; his only question was whether Monera or Prokaryota should be used for the new kingdom. In 1968, he proposed Prokaryotae as a taxon “at the highest level” and described it as “a kingdom of microbes... characterized by the possession of nucleoplasm devoid of basic protein and not bounded from cytoplasm by a nuclear membrane.” He suggested Eukaryotae as a possible taxon at the same level to include other protists, plants, and animals [35]. The following year, A. Allsopp, at the University of Manchester, suggested

that Prokaryota and Eukaryota be given the status of “superkingdom” [1]. When, in 1973, Stanier and Lwoff returned to catalogue the differences between prokaryotes and eukaryotes, they assigned both to Haeckel’s kingdom of Protists, just as Stanier and van Niel had done in 1962 [51,53].

Two bacterial urkingdoms

Molecular biology offered new approaches to phylogeny. Instead of comparative anatomy and physiology, one could construct phylogenetic trees comparing the sequence divergence of genes or proteins. Genetic mutations that either have no effect or that improve protein function would accumulate over time. As two species diverge from an ancestor, the sequences of the genes they share also diverge, and, as time advances, the genetic divergence will increase. Frederick Sanger and his colleagues had succeeded in determining the complete sequence of insulin in 1955 [39]. The message for evolutionary biology was noted by Francis Crick in 1958:

Biologists should realize that before long we shall have a subject which might be called ‘protein taxonomy’—the study of amino acid sequences of proteins of an organism and the comparison of them between species. It can be argued that these sequences are the most delicate expression possible of the phenotype of an organism and that vast amounts of evolutionary information may be hidden away within them [13].

Studies of molecular evolution were underway in the 1960s, based on amino-acid sequence comparisons of various proteins, including hemoglobin, cytochrome *c*, and ferredoxin [47]. The key to a phylogenetic classification of bacteria lay in choosing the right molecules, those that were central to the functioning of all known life on earth. Emile Zuckerkandl and Linus Pauling had pioneered the use of hemoglobin sequences to infer primate phylogeny [75]. Walter Fitch and Emmanuel Margoliash compared amino-acid sequences of cytochrome *c* to infer phylogenetic relationships among diverse eukaryotes, from horses, humans, pigs, rabbits, chickens, tuna, and baker’s yeast [15].

To construct a deep bacterial phylogeny, Carl Woese, at the University of Illinois, looked to the translation machinery—not to proteins, but to those RNAs that, together with proteins, comprise ribosomes, the organelles in which translation from nucleic acid to protein occurs in all organisms. The basic techniques for his project were announced in 1965, when Sanger and his co-workers published a method for sequencing and cataloguing short RNA nucleotide chains [40].

Beginning in 1970, Woese focused on oligonucleotide (short fragments of 5–20 nucleotides) comparisons of 16S ribosomal RNA. Using that probe, he and his colleagues declared, in 1977, that they had discovered a new form of life, the archaeobacteria, and with it they challenged the dichotomous prokaryote–eukaryote view of life [69]. The archaeobacterial concept grew out of Woese's interest in the origin of the genetic code and the evolution of the translation machinery. Indeed, he had already challenged one aspect of the prokaryote–eukaryote dualism 7 years earlier, when he suggested that prokaryotes did not lead to eukaryotes. The prokaryote–eukaryote dichotomy had been founded largely on structural differences discernable by the electron microscope, but Woese began to conceptualize it at the molecular genetic level. Based on differences in their translation apparatus, he argued that both lineages may have emerged from a more primitive life form in the throws of evolving its translation machinery [64]. Woese and his post-doctoral student George Fox later named the hypothetical precellular entities “progenotes” [65,69].

By 1980, the 16S rRNA of almost 200 species of bacteria and eukaryotes had been characterized. Those results often showed that the taxonomic groups that *Bergey's Manual* distinguished were not genealogically coherent groups; instead, they were paraphyletic or polyphyletic [16]. No claim based on the 16S rRNA approach attracted more interest than the group Woese and Fox called “archaeobacteria” [69].

In June of 1976, Woese had requested advice from many microbiologists about what organisms to analyze. One of his colleagues at the University of Illinois, Ralph Wolfe, suggested that he examined an odd group of methane-producing bacteria. Methanogens are chemo-autotrophs; they derive their energy from carbon dioxide and hydrogen. By the time Woese's laboratory tested the methanogens, he had created a list of oligonucleotides characteristic of prokaryotic 16S rRNA. The methanogens were missing almost all those signature sequences. Woese and Fox named the new form of life, or “urkingdom,” “archaeobacteria” to distinguish them from true bacteria or “eubacteria” [69]

Against adaptationism

Over the next 2 years, Woese's laboratory expanded the archaeobacterial urkingdom to include other organisms that were known to live in extreme environments: extreme halophiles found in brines five times as salty as the oceans, and thermophiles, *Sulfolobus* and *Thermoplasma*, found in extreme geothermal environments. All of these organisms were shown to have unusual traits in common: their mem-

brane lipids were different from those of other bacteria, the structural components of their cell walls were different, and so were their transcription enzymes.

By the 1970s, lipid specialists had noted that the structures of lipids in the membranes of extreme halophiles, *Sulfolobus* and *Thermoplasma*, were different from those of typical bacteria. But those odd membranes were thought to be adaptations to extreme environments; thus, they were considered to be convergent properties, not ancient traits in common to what otherwise appeared to be unrelated and phenotypically very different organisms. The lipids were definitely not considered to be of any phylogenetic significance, as Thomas Brock commented in 1978: “The fact that *Sulfolobus* and *Thermoplasma* have similar lipids is of interest, but almost certainly this can be explained by convergent evolution. This hypothesis is strengthened by the fact that *Halobacterium*, another quite different organism, also has lipids similar to those of the two acidophilic thermophiles” [6]. In light of the 16S rRNA comparisons, Thomas Tornabene and Thomas Langworthy, working at Colorado State University, showed that the same unusual lipids were found in methanogens as well, and even among those methanogens that lived in moderate temperatures. Those findings supported the concept that these unusual organisms shared a deep phylogenetic lineage, as indicated by 16S rRNA sequence analysis. As Tornabene and Langworthy concluded in an article in *Science* in 1979:

The data reported here establish that the mesophilic methanogens also contain the same ether lipids, which must represent a long evolutionary relationship between methanogens, *Halobacterium*, *Thermoplasma*, and *Sulfolobus*; this argues against the possibility these ether lipids reflect environmental adaptation [54].

The cell-wall chemistry of these organisms was also shown to be of special phylogenetic importance. In 1977, Otto Kandler, at the University of Munich, reported that the cell walls of methanogens lacked the complex molecule peptidoglycan (or murein) typical of bacteria (and indeed part of Stanier and van Niel's definition of “prokaryotes”), just as did the cell walls of halophiles [23]. He and his collaborators subsequently showed that the same was true of the other organisms that Woese's lab had grouped together as archaeobacteria [24]. Kandler became a key champion of the archaeobacteria concept. At the Max-Planck Institute in Martinsried, a suburb of Munich, Kandler's former student Karl Stetter and Wolfram Zillig reported that the structure of transcription enzymes, the DNA-dependent RNA polymerases of *Halobacterium halobium*, *Sulfolobus acidocaldarius*, and *Metha-*

nobacterium differed characteristically from their counterparts in typical bacteria and closely resembled the transcription enzymes of eukaryotes [72,73,74].

Woese and Fox challenged the prokaryote concept the moment they named the new form of life “archaebacteria” [69]. The prokaryote concept, they argued, concealed more than it revealed. As they put it in 1977, “[d]ividing the living world into Prokaryotae and Eukaryotae has served, if anything, to obscure the problem of what extant groupings represent the various primeval branches from the common line of descent. The reason is that eukaryote/prokaryote is not primarily a phylogenetic distinction, although it is generally treated so [69].” In a landmark paper in *Science* in 1980 [16], titled “The phylogeny of prokaryotes,” Woese and his collaborators again argued that prokaryote–eukaryote was a false dichotomy, a phylogenetic deception, and a fundamental obstacle to understanding the evolution of life. In their scheme, prokaryotes did not lead to the eukaryotes, as they did in previous models. Instead, all three lineages diverged early from proto-cells, “progenotes,” which were in the throes of evolving their translation mechanisms both in terms of precision and speed [16,65]. Archaebacteria were no more related to eubacteria than either were to eukaryotes. The characteristic differences between archaebacterial and eubacterial cell walls, membranes, and transcription enzymes suggested that these features were in the process of development at the progenote stage of evolution [16].

To further emphasize that prokaryotes do not have a common ancestry and to counter the notion that the archaebacteria are “just bacteria,” in 1990, Woese, Kandler, and Mark Wheeler renamed them the Archaea [70]. In doing so, they made a formal taxonomic proposal of three “domains” of life: Bacteria, Archaea, and Eukarya. For Woese and his colleagues, the differences in cell-wall structure, translation processes, and other characteristics that distinguished Bacteria and Archaea, combined with the rRNA data indicating fundamental phylogeny differences between them, effectively refuted the concept of the prokaryote in both the organizational and the phylogenetic sense of this term [66,67].

Microbial phylogenetics grew in the 1980s, as techniques for sequencing RNA and DNA dramatically improved [33,41]. The discovery of the archaebacteria heralded the great depth and diversity to be explored in the microbial world. The three domains, or superkingdoms, were widely accepted and adopted in textbooks. Still, the concepts of Woese and his co-workers remained contentious. Few microbial phylogeneticists accepted the trilogy as a replacement for the prokaryote–eukaryote dichotomy [58], nor did they accept that three lineages had emerged independently from a hypothetical progenote in the throws of developing the translation mechanism.

Polygenomic chimeras

Is Eukarya real? Does the eukaryote have an independent lineage comparable to Archaea and Bacteria? Woese, Fox, and their collaborators had raised that issue in 1980 [16]. Perhaps the nucleus of the eukaryote had emerged from a chimeric mixture of eubacterial and archaebacterial genes, and had no lineage of its own. By that time, the 16S rRNA technology already indicated that mitochondria and chloroplasts were indeed symbionts.

When Stanier and van Niel defined the bacterium in 1962, they understood the organization of the prokaryote and eukaryote in terms of a single genome: they made no mention of plasmids in bacteria or of organellar heredity in eukaryotes. That year, DNA was demonstrated in chloroplasts, and the following year in mitochondria. Those organelles also had their own transcription machinery distinct from that of the nucleus. The concept that mitochondria and chloroplasts had arisen as engulfed symbionts in the remote past had persisted on the edge of biology throughout the 20th century, but like bacterial phylogeny itself had been dismissed as idle speculation [46]. The idea re-emerged and was championed by Lynn Margulis [29]. However, it still remained possible that those organelles emerged endogenously by differentiation within the cell. Their similarities with bacteria would simply be a case of convergent evolution. That possibility was fortified with evidence indicating that most of the genes controlling crucial mitochondria and chloroplast functions were in the nucleus.

rRNA technology offered a means for resolving the issue. In the mid-1970s, this technology was exported by Woese’s technician, Linda Bonen, from Urbana to the laboratories of Ford Doolittle and Michael Gray, at Dalhousie University, Halifax, Canada. Doolittle’s and Woese’s laboratories focussed on chloroplast rRNA [2,4,14]; Gray’s focussed on mitochondrial rRNA [3,17]. Collectively, their results indicated that the origins of chloroplasts and mitochondria were independent from each other and from nuclear-derived rRNA. Chloroplasts were descendants of the photosynthetic blue-green bacteria (cyanobacteria); the mitochondrial ancestor was traced to the α -proteobacteria [18]. There were no comparable data to test whether centrioles/kinetosomes arose as symbionts, as Margulis suggested [29]. The evidence for DNA in centrioles/kinetosomes had been on-again, off-again since the 1960s, and was effectively refuted in the 1990s [19,45].

What about the eukaryotic cell nucleus? Did it also have a symbiotic origin? The origin of the eukaryotic cell nucleus emerged as one of the pressing problems of microbial phylo-

genetics. The issue concerned the kinds of genes found in the nucleus. The nuclear genome seemed to comprise three phylogenetically distinct groups of genes: (i) information-transfer genes concerned with transcription and translation and closely related to those of Archaea; (ii) eubacterial genes thought to be transferred to the nucleus from the mitochondria, and (iii) eubacterial genes whose functions were not obviously related to mitochondria [38].

Several interpretations have been offered [38]: “non-mitochondrial” genes could have been acquired from symbionts and lost after the emergence of eukaryotes; they could have been acquired by lateral gene transfer before [65] or after the emergence of the eukaryotic lineage, by symbiosis between an archaeobacterium and an extinct third form of life. One of the most common hypotheses is that the nucleus emerged from an engulfed archaeobacterial symbiont in a eubacterial host [26, 38]. Alternatively, many of the ancient “non-mitochondrial” genes in the present eukaryotic nucleus were actually derived from the ancestor of mitochondria, which had entered an archaean that subsequently evolved the nucleus [31]. In these last two models, the Eukarya would not represent a lineage comparable to Archaea and Bacteria.

Embraced by the Prokaryote

The concept of the Archaea as a superkingdom or domain on par with eukaryotes has also been challenged. Critics have argued that the differences between the archaeobacteria and the eubacteria pale when the structure of bacteria (*sensu lato*) is compared to the structure of eukaryotes [30,34]. Nor does everyone see the value and necessity of maintaining a phylogenetic basis for taxonomy when the degree of evolutionary change is considered to be so great. Indeed, although phylogeny is acknowledged as the preferred method of grouping bacteria, few taxonomists have abandoned the venerable prokaryote–eukaryote dichotomy. As noted in the five-kingdom proposal of the late 1960s, monophyly (though valued) was not considered to be a requirement for all of those who partitioned nature’s kingdoms. In accordance with such standard reference volumes as *Bergey’s Manual* and *The Prokaryotes* [30], there is support for the superkingdoms (or empires [34]) *Eukaryotae* and *Prokaryotae*, with Archaea and Bacteria placed as kingdoms within the latter.

In the arguments for a dichotomous world view, the history of the concepts prokaryote and eukaryote has been repeatedly confused and mythologized [43] as, for example, in Ernst Mayr’s [34] assertion that “Stanier and van Niel recognized two empires, the prokaryotes and the eukaryotes.” As emphasized here and elsewhere [43], the prokar-

yote–eukaryote dichotomy, as originally constructed by Stanier and van Niel, was decidedly not meant to be a taxonomic distinction. It was meant to close once and for all a controversy about the structure of bacteria which had lasted for at least 80 years: Did prokaryotes have a nucleus? Did blue-green algae have plastid-like entities? How could one distinguish bacteria from viruses? The prokaryote was an organizational concept, not a phylogenetic one, though it was given imperial status shortly thereafter as an ancient kingdom of organisms that preceded eukaryotes. As such, the prokaryote concept was confronted soon after the emergence of molecular approaches to bacterial phylogenetics, as 16S rRNA probes revealed the group to comprise two lineages conceived as being of equal phylogenetic status.

Today, the three domains are widely accepted, and “prokaryotes” are generally recognized not to represent a monophyletic group. Still, whether the term should remain in biology is hotly debated among microbial phylogeneticists. Woese and Norman Pace insist the word should be expunged from the biological lexicon, because of its false and misleading premise concerning monophyly and a negative organizational definition [37, 68]. Others, who recognize the fundamental phylogenetic trilogy of the primary domains and emphasize that the genetic informational systems of Archaea and Bacteria differ radically in terms of replication, transcription, and translation, still defend the term prokaryote on organizational grounds and see no fault with a negative definition. While some of the venerable morphological issues and confusions remain in the definition of prokaryote, other molecular features have been offered to organizationally conjoin the Archaea and Bacteria.

Whether or not the bacterium contains a nucleus is only a matter of definition. Stanier and van Niel [51] defined prokaryote as possessing a nucleus that multiplies by simple fission, not by mitosis. Today it is typically stated that the prokaryote lacks a nucleus [32]. But while bacteria lack a nuclear membrane, they do possess a “nuclear body” or “nucleoid” where DNA is localized. Both Bacteria and Archaea lack the structural complexity of the eukaryotic cell, as expressed in its cytoskeleton and membrane-enclosed organelles. Walsh and Doolittle [62] also pointed to several common molecular features of “the prokaryotic domains,” especially the lack of spliceosomal introns, which are central to eukaryotic cell differentiation and complexity. Bacteria and Archaea are characterized by: “a typically (but not always) circular chromosome(s); absence of spliceosomal introns; organization of many genes into operons (sometimes with homologous genes in the same order)”. Perhaps the key positive common character that unites prokaryotes, Martin and Koonin added, is the coupling of transcription and translation [32].

Thus, the concept of the bacterium continues to be reconfigured in organizational terms regardless of phylogenetic considerations, and the dichotomous world view of prokaryote and eukaryote continues to frame much of biological thinking. Yet, it is ironic that the transcription and translation mechanisms considered to be the key common feature of the prokaryote are, as mentioned earlier, understood by all to be radically different in Archaea and Bacteria at the molecular level. Together with hundreds of other signature gene clusters, they are held by Woese and his collaborators as defining those differences in the cell's "design fabric" that wrought the fundamental divergence of life into three domains [17]. What ought to define bacterial organization, it seems, is as unresolved today as it was before Stanier and van Niel's prokaryote concept.

References*

- Allsopp A (1969) Phylogenetic relationships of the procaryota and the origin of the eucaryotic cell. *New Phytol* 68:591-612; p 607
- Bonen L, Doolittle WF (1975) On the prokaryotic nature of red algal chloroplasts. *Proc Natl Acad Sci USA* 72:2310-2314
- Bonen L, Cunningham RS, Gray MW, Doolittle WF (1977) Wheat embryo mitochondrial 18S ribosomal RNA: evidence for its prokaryotic nature. *Nucleic Acids Res* 4:663-671
- Bonen L, Doolittle WF (1976) Partial sequences of 16S rRNA and the phylogeny of blue-green algae and chloroplasts. *Nature* 261:669-673
- Breed RS, Murray EGD, Hitchens PA (1948) *Bergey's manual of determinative bacteriology*, 6th edn. The Williams and Wilkins Co., Baltimore, MD
- Brock T. (1978) *Thermophilic microorganisms and life at high temperatures*. Springer-Verlag, Heidelberg
- Chatton E (1925) *Pansporella perplexa*, réflexions sur la biologie et la phylogénie des Protozoaires. *Ann Sci Nat Biol Anim Zool*, 10e série, VII, 6-84; p 76 (In French)
- Cohn F (1875) *Untersuchungen über Bacterien II. Beiträge zur Biologie der Pflanzen* 1:141-207
- Copeland E B (1927) What is a plant? *Science* 65:388-390
- Copeland HF (1938) The kingdoms of organisms. *Quart Rev Biol* 13:383-420; p 386
- Crick F H C (1958) The biological replication of macromolecules. *Symp Soc Exptl Biol* 12:138-163
- Charles D (1859) *On the origin of species*. With an introduction by Ernst Mayr, Facsimile edition of 1859. Harvard University Press 1964, Cambridge, MA
- Dobell CC (1911) Contributions to the cytology of the bacteria, *Quart J Microsc Sci* 56:395-506
- Doolittle WF, Woese CR, Sogin ML, Bonen L, Stahl D (1975) Sequence studies on 16S ribosomal RNA from a blue-green alga. *J Mol Evol* 4:307-315
- Fitch WM, Margoliash E (1967) The construction of phylogenetic trees—a generally applicable method utilizing estimates of the mutation distance obtained from cytochrome *c* sequences. *Science* 155:279-284
- Fox GE, Stackebrandt E, Hespell RB, Gibson J, Maniloff J, Dyer TA, Wolfe RS, Balch WE, Tanner RS, Magrum LJ, Zablen LB, Blakemore R, Gupta R, Bonen L, Lewis BJ, Stahl DA, Luerhsen KR, Chen KN, Woese CR (1980) The phylogeny of prokaryotes. *Science* 209:457-463
- Graham DE, Overbeek R, Olsen GJ, Woese CR (2000) An archaeal genomic signature. *Proc Natl Acad Sci USA* 97:3304-3308
- Gray MW, Doolittle WF (1982) Has the endosymbiont hypothesis been proven? *Microbiol Rev* 46:1-42
- Hall JL, Luck DJ (1995) Basal body-associated DNA: *In situ* studies in *Chlamydomonas reinhardtii*. *Proc Natl Acad Sci USA* 92:5129-5133
- Haeckel E (1866) *Generelle Morphologie der Organismen* 2 vols. George Reimer, Berlin
- Haeckel E (1904) *The wonders of life. A popular study of biological philosophy*, translated by Joseph McCabe. Harper and Brothers, pp 195-196
- Henrici AT (1939) *The biology of bacteria*. Heath and Co, New York, NY
- Kandler O, Hippe H (1977) Lack of peptoglycan in the cell walls of *Methanosarcina barkeri*. *Arch Microbiol*. 113: 57- 60
- Kandler O (1994) Cell wall biochemistry and the three domain concept of life. *System Appl Microbiol* 16:501-509
- Kluyver A J, van Niel CB (1936) Prospects for a natural system of classification of bacteria. *Zentralbl Bakteriell Parasitenkd Infektionskr Hyg Abt II* 94:369-402
- Lake, J (1988) Origin of the eukaryotic nucleus detected by rate-invariant analysis of rRNA. *Nature* 331:184-186
- Lederberg J, Tatum EL (1946) Novel genotypes in mixed cultures of biochemical mutants of bacteria. *Cold Spring Harbor Symp Quant Biol* 11:113-114
- Lwoff A (1957) The concept of virus. *J Gen Microbiol* 17:239-253
- Margulis L (1981) *Symbiosis in cell evolution*, 1st edn. WH Freeman, New York, NY
- Margulis L, Guerrero R (1991) Kingdoms in turmoil. *New Scientist* 23:46-50
- Martin W, Muller M (1998) The hydrogen hypothesis for the first eukaryote. *Nature* 392:37-41
- Martin W, Koonin EV (2006) A positive definition of prokaryotes. *Nature* 442:868
- Maxam AM, Gilbert W (1977) A new method for sequencing DNA. *Proc Natl Acad Sci USA* 74: 560-564
- Mayr E (1998) Two empires or three? *Proc Natl Acad Sci USA* 95:9720-9723
- Murray RGE (1968) Microbial structure as an aid to microbial classification and taxonomy. *SPISY (Faculté des Sciences de l'Université J. E. Purkyne Brno)* 34:249-252
- Murray RGE 1962 to R.Y. Stanier. *Natl Archives Canada*, MG 31, Accession J35, vol 6
- Pace N (2006) Time for a change. *Nature* 441:289
- Roger A (1999) Reconstructing early events in eukaryotic evolution. *Am Natural* 154: S146-S163
- Sanger F, Thompson EO, Kitai R (1955) The amide groups of insulin. *Biochem J* 59:509-518
- Sanger, F, Brownless GG, Barrell G (1965) A two-dimensional fractionation procedure for radioactive nucleotides. *J Mol Biol* 13:373-398
- Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA* 74:5463-5467
- Sapp J (2005) Mitochondria and their host: morphology to molecular phylogeny. In Martin W, Müller M (eds) *Mitochondria and hydrogenosomes*. Springer Verlag, Heidelberg
- Sapp J (2005) The prokaryote-eukaryote dichotomy: meanings and mythology. *Microbiol Mol Biol Rev* 69:292-305
- Sapp J (2005) The bacterium's place in nature. In: Sapp J (ed) *Microbial phylogeny and evolution*. Oxford University Press, New York, NY; pp 1-52
- Sapp J (1998) Freewheeling centrioles. *Hist Philos Life Sci* 20:255-290
- Sapp J (1994) *Evolution by association. A history of symbiosis*. Oxford University Press, New York, NY

*Note: Due to the nature of the above article, some of the references are not formatted in the style followed by the journal.

47. Schwartz R M, Dayhoff MO (1978) Origins of prokaryotes, eukaryotes, mitochondria, and chloroplasts. *Science* 199:395-403
- 47bis. Soyer-Gobillard MO (2006) Edouard Chatton (1883–1947), life and work and the dinoflagellate protists: concepts and models. *Int Microbiol* 9:173-177
48. Stanier RY (1961) La place des bactéries dans le monde vivant. *Ann Inst Pasteur* 101 no. 3 297-312
49. Stanier RY to Murray RGE, May 21, 1962 National Archives of Canada, MG 31, Accession J35, vol 6
50. Stanier RY, van Niel CB (1941) The main outlines of bacterial classification. *J Bacteriol* 42:437-466
51. Stanier RY, van Niel CB (1962) The concept of a bacterium. *Arch Mikrobiol* 42:17-35
52. Stanier R, Doudoroff M, Adelberg E (1963) *The microbial world*, 2nd edn. Englewood Cliffs, Prentice-Hall, NJ
53. Stanier R, Lwoff A (1973) Le concept de microbe de Pasteur à nos jours. *Nouvelle Press Médicale* 2:1191-1198
54. Tornabene TG, Langworthy TA (1979) Diphytanyl and dibiphytanyl glycerol ether lipids of methanogenic archaeobacteria. *Science* 203:51-53
55. van Niel CB to Stanier R, August 13, 1941. Stanier papers, National Archives of Canada, Ottawa
56. van Niel CB (1946) The classification and natural relationships of bacteria. *Cold Spring Harbor Symp Quant Biol* 11:285-301
57. van Niel CB (1955) Classification and taxonomy of the bacteria and blue green Algae. In: Babcock EB, Durham JW, Myers GS (eds) *A century of progress in the natural sciences 1853-1953*. California Academy of Sciences, San Francisco, pp 89-114
58. Walsh DA, Doolittle VF (2005) The real domains of life. *Curr Biol* 15:R237-240
59. Whittaker R. (1957) The kingdoms of the living world. *Ecology* 38:536-538
60. Whittaker RH (1959) On the broad classification of organisms. *Quart Rev Biol* 34:210-226
61. Whittaker RH (1969) New concepts of kingdoms of organisms. *Science* 163:150-163
62. Wilson EB (1900) *The cell in development and inheritance*. Mcmillan, New York
63. Winogradsky S (1952) Sur la classification des bactéries. *Ann Inst Pasteur* 82:125-131
64. Woese CR (1970) The genetic code in prokaryotes and eukaryotes. In: Charles HP, Knight BCJG (eds) *Organization and control in prokaryotic and eukaryotic cells*. 20th Symp Soc Gen Microbiol, Cambridge University Press, Cambridge, UK, pp 39-54
65. Woese CR (1998) The universal ancestor. *Proc Natl Acad Sci USA* 95:6854-6859
66. Woese CR (1998) Default taxonomy: Ernst Mayr's view of the microbial world. *Proc Natl Acad Sci USA* 95:11043-11046
67. Woese CR (2004). A new biology for a new century. *Microbiol Mol. Biol Rev* 68:173-186
68. Woese CR, Fox GE (1977) The concept of cellular evolution. *J Mol Evol* 10:1-6
69. Woese CR, Fox GE (1977) Phylogenetic structure of the prokaryotic domain: the primary kingdoms. *Proc Natl Acad Sci USA* 74:5088-5090
70. Woese CR, Kandler O, Wheelis ML (1990) Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. *Proc Natl Acad Sci USA* 87:4576-4579
71. Zablen LB, Kissil MS, Woese CR, Buetow DE (1975) Phylogenetic origin of the chloroplast and prokaryotic nature of its ribosomal RNA. *Proc Natl Acad Sci USA* 72: 2418-2422
72. Zillig W, Schnabel R, Tu J, Stetter KO (1982) The phylogeny of archaeobacteria, including novel anaerobic thermoacidophiles in the light of RNA polymerase structure. *Naturwissenschaften* 69:197-204
73. Zillig W, Stetter KO, Tobien M (1978) DNA-dependent RNA polymerase from *Halobacterium halobium*. *Eur J Biochem* 91:193-199
74. Zillig W, Stetter KO, Janekovic D (1979): DNA-dependent RNA polymerase from the archaeobacterium *Sulfolobus acidocaldarius*. *Eur J Biochem* 96:597-604
75. Zuckerkandl E, Pauling L (1965) Molecules as documents of evolutionary history. *J Theor Biol* 8:357-366

La doble cara del concepto de procariota

Resumen. Hasta que en 1962 Roger Y. Stanier y C.B. van Niel publicaron su famoso artículo "The concept of a bacterium", las bacterias habían carecido de definición. La articulación de la dicotomía procariota-eucariota representó un momento crucial en la historia de la biología. La finalidad de esta revisión es ofrecer una breve perspectiva del contexto en el cual hizo aparición satisfactoriamente el concepto de procariota en la década de 1960 y de lo que iba a significar. En aquel tiempo, se incluían dos conceptos en la dicotomía procariota-eucariota. Uno, de tipo organizativo, se refería a la estructura celular comparativa; el otro, filogenético, se refería a una clasificación natural. Este trabajo describe cómo ambos conceptos se hicieron inseparables; cómo el concepto procariota llegó a significar un grupo monofilético que precedió a los eucariotas, y cómo se mantuvo indiscutible este enfoque durante 15 años, hasta el nacimiento de la biología molecular evolutiva y el desarrollo de métodos coherentes en filogenética bacteriana basados en el rRNA 16S. Hoy en día, si bien los expertos en filogenia microbiana están generalmente de acuerdo en que los procariotas forman un grupo polifilético, no hay acuerdo sobre si el término debería mantenerse en un sentido organizativo. [*Int Microbiol* 2006; 9(3):163-172]

Palabras clave: procariota · eucariota · filogenia · evolución · taxonomía

As duas faces do conceito de procariota

Resumo. Até que em 1962 Roger Y. Stanier e C. B. van Niel publicassem seu famoso artigo "The concept of a bacterium", as bactérias careciam de definição. A articulação da dicotomia procariota-eucariota supôs um momento vital na história da biologia. A finalidade desta revisão é oferecer uma breve perspectiva do contexto no qual fez satisfatoriamente sua aparição o conceito de procariota na década de 1960 e no seu significado. Naquele tempo, dois conceitos foram incluídos na dicotomia procariota-eucariota. Um, de tipo organizativo, se referia à estrutura celular comparativa; o outro, filogenético, se referia à classificação natural. Este trabalho descreve como ambos conceitos tornaram-se inseparáveis; que a célula procariota vinha a significar um grupo monofilético que precedeu aos eucariotas, e que este enfoque se manteve inalterado durante 15 anos, até o nascimento da biologia molecular evolutiva e o desenvolvimento de métodos coerentes da filogenética bacteriana baseados no rRNA 16S. Hoje em dia, os estudiosos em filogenia microbiana estão geralmente de acordo que os procariotas formam um grupo polifilético, no entanto, não há um consenso se o termo deveria manter-se em um sentido organizativo. [*Int Microbiol* 2006; 9(3):163-172]

Palavras chave: procariota · eucariota · filogenia · evolução · taxonomia